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Research Note

Prevalence and Antimicrobial Resistance Profiles of *Escherichia coli* O157:H7 and *Salmonella* Isolated from Feedlot Lambs†

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ABSTRACT

The present study examined the incidence of *Escherichia coli* O157:H7 and *Salmonella* in feedlot lambs. Fifty-six feedlot lambs from eight sheep farming operations were grouped in a single drylot pen, fed, and managed as is typical in the southwestern United States. Fecal samples were collected on days 0, 46, 87, and 122 of the feeding period via rectal palpation. Wool samples (ventral midline) were collected one time only at the feedlot, immediately prior to shipping to the processing plant, and carcass swabs were collected following slaughter. All samples were cultured for *E. coli* O157:H7, *Salmonella,* and fecal coliforms, and select isolates were examined for antimicrobial susceptibility. Overall, the percentages of fecal and wool samples positive for *E. coli* O157:H7 averaged 9 and 18%, respectively. One carcass swab was *E. coli* O157:H7 positive. Of the 155 fecal samples collected, 11 (7%) were *Salmonella* positive. *Salmonella* was detected in nearly 50% of the wool samples collected prior to slaughter, while none of the carcasses were *Salmonella* positive 24 h postslaughter. All isolates (*E. coli* O157:H7, *Salmonella,* and fecal coliforms) were susceptible to ceftiofur, enrofloxacin, and trimethoprim-sulfamethoxazole. One *E. coli* O157:H7 isolate cultured from a carcass swab was resistant to seven antibiotics, and seven wool *E. coli* O157:H7 isolates were multidrug resistant. Results of this research demonstrate that feedlot sheep are naturally colonized with *E. coli* O157:H7 and *Salmonella* and wool can be a source of carcass contamination; however, in-plant processing procedures and intervention strategies were largely effective in preventing carcass contamination.

Verocytotoxin-producing *Escherichia coli* O157:H7 was recognized as a major foodborne pathogen in 1982 following two food-associated outbreaks of unusual gastrointestinal illness *(8).* This organism is now recognized as an important causative agent of foodborne disease, having been implicated in several outbreaks in the United States, Canada, and the United Kingdom *(8).* Ruminants are considered to be an important reservoir of *E. coli* O157:H7, and sheep, like cattle, are naturally colonized by this pathogen *(6).*

Salmonella is one of the most important of the foodborne pathogens worldwide and is reported to colonize virtually all animals, including humans, livestock, reptiles, birds, and rodents *(14).* An estimated 1.4 to 3 million cases of salmonellosis occur in the United States each year, with more than 500 deaths *(3). Salmonella* infections in humans are usually acquired via consumption of contaminated food; however, direct contact with infected animals can result in human infection *(1).* As they are for *E. coli* O157:H7, ruminants are reservoirs for *Salmonella,* often appearing asymptomatic while shedding this pathogen into the environment *(11, 15).* While sheep have been reported to be carriers of *Salmonella,* prevalence data in the United States are limited *(9).* The objectives of the present research were to examine *Salmonella* and *E. coli* O157:H7 prevalence in feedlot sheep throughout the feeding period and following slaughter.

MATERIALS AND METHODS

Animals and sample collection. Fifty-six feedlot lambs (fine wool, hair, and crossbred; wethers and ewes) originating from eight different sheep farming operations were utilized in the study. Lambs entered the feedlot at the same time, were maintained together in a single drylot pen, and were fed and managed as is typical for a sheep feedlot in the southwestern United States. Lambs were shipped to slaughter based on their body weight; therefore, slaughter dates are staggered and most lambs were sampled (feces) on multiple occasions.

Fecal samples (approximately 20 g) were collected from all lambs on days 0, 46, 87, and 122 of the feeding period during the summer and fall of 2003 via rectal palpation. Wool samples were collected one time only at the feedlot, immediately prior to shipping to the processing plant. Wool was shorn from an area

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approximately 300 cm^2 on the ventral midline, and the clippers were cleaned and disinfected between samples. Following slaughter, carcass swabs were collected using Speci-sponge bags (Whirl-Pak, Nasco, Modesto, CA) with each sponge moistened with sterile phosphate-buffered saline (PBS) immediately prior to use. Three sites (leg, shoulder, and bung/hock region) were swabbed on each carcass (one sponge per carcass) following 24 h in the cooler. All samples were shipped overnight to the laboratory in College Station, TX, for bacterial culture as described below.

Bacterial culture and isolation. All samples were cultured for *E. coli* O157:H7 and *Salmonella* within 24 h of collection. *E. coli* O157:H7 culture and isolation were conducted using an immunomagnetic separation technique as described previously *(10)*. Briefly, 10 g of feces was enriched in 90 ml of gram-negative broth containing vancomycin (8 μg/ml), cefixime (0.5 μg/ml), and cefsoludin (10 μg/ml) and incubated (6 h, 37°C). Following incubation, 20 μl of anti–*E. coli* O157:H7 antibody-labeled paramagnetic beads (Neogen Corp., Lansing, MI) was added to 1-ml volumes of the above enrichments, mixed, and washed. Fifty microliters of the resulting suspension was spread plated on CHROMagar O157 (DRG International, Mountain Side, NJ) plates (containing 2.5 μg/ml potassium tellurite) and incubated overnight (37°C). Pink colonies exhibiting typical *E. coli* O157:H7 morphology were resuspended in PBS (pH 6.5) and confirmed as *E. coli* O157:H7 using the Reveal microbial screening test according to the manufacturer's instructions (Neogen Corp.). Wool (approximately 10 g) and carcass swab sponges were enriched in 20 ml of sterile 22% brilliant green bile broth and incubated (6 h, 37°C). Following incubation, all culture techniques were the same as described above for fecal samples. *E. coli* O157:H7 isolates were stored (−80°C) using CryoCare bacterial preservers (Key Scientific Products, Round Rock, TX).

Salmonella was cultured by enriching approximately 10 g of feces in 90 ml of tetrathionate broth (37°C, 24 h), followed by a second enrichment in Rappoport-Vassilidis broth (100 μl in 5 ml, 42°C, 24 h). Enrichments were plated on brilliant green agar (Oxoid Ltd., Hampshire, UK) supplemented with novobiocin (25 μg/ml) and incubated (37°C, 24 h). Wool and carcass swabs were enriched in 20 ml of buffered peptone water and incubated (37°C, 24 h) prior to secondary and tertiary enrichments and plating as described above for fecal samples. Following incubation, colonies exhibiting typical *Salmonella* morphology were confirmed biochemically using lysine and triple sugar iron agars. Positive samples were restreaked on tryptic soy agar with 5% sheep blood (Becton Dickinson and Company, Franklin Lakes, NJ) for further confirmation, and serogrouping was conducted using slide agglutination with *Salmonella* antiserum (Becton Dickinson and Company). *Salmonella* isolates were stored (−80°C) as above.

Fecal coliforms were isolated from CHROMagar O157 plates based on color and resuspended in PBS and frozen as above. Unless noted otherwise, all reagents and antibiotics were obtained from Sigma Chemical Co. (St. Louis, MO).

Antimicrobial susceptibility testing. One isolate from each *E. coli* O157:H7–positive sample ($n = 26$) and each *Salmonella*-positive sample ($n = 35$) and one fecal coliform isolate from the 20 fecal samples at each collection ($n = 80$) were examined for antimicrobial susceptibility by using the Sensititre automated antimicrobial susceptibility system according to the manufacturer's directions (Trek Diagnostic Systems, Westlake, OH). Broth microdilution was used according to the methods described by the Clinical and Laboratory Standards Institute *(5)* utilizing the bovine/porcine isolate susceptibility testing panels to determine MICs for the following antimicrobials: ampicillin, apramycin, ceftiofur, chlortetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, sulfachloropyridazine, sulfadimethoxine, sulfathiazole, and trimethoprim-sulfamethoxazole. Resistance breakpoints were determined using the NCCLS interpretive standards *(5)*. *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Enterococcus faecalis* ATCC 29212 were used as quality control organisms.

TABLE 1. *Prevalence of* E. coli *O157:H7 and* Salmonella *in fecal, wool, and carcass swab samples from feedlot sheep*

Farm	No. of lambs	No. of samples positive/total no. of samples					
		E. coli O157:H7			*Salmonella*		
		Feces	Wool	Carcass	Feces	Wool	Carcass
A	7	0/21	1/7	1/7	0/16	4/7	0/7
B	7[a]	0/23	1/6	0/6	0/21	2/6	0/6
C	4[a]	0/12	1/3	0/3	0/11	1/3	0/3
D	7[a]	1/23	1/6	0/6	4/22	3/6	0/6
E	8	10/27	0/8	0/8	1/25	4/8	0/8
F	9	4/31	2/9	0/9	3/29	5/9	0/9
G	8[a]	0/23	2/7	0/7	2/19	4/7	0/7
H	6[a]	0/12	1/5	0/5	1/12	2/5	0/5
Overall	56	15/162	9/50	1/51	11/155	25/51	0/51

[a] One lamb died or was removed from the pen due to poor performance.

RESULTS

Results of the bacterial culture for *E. coli* O157:H7 and *Salmonella* are grouped across the collection period and presented by farm in Table 1. Sheep with fecal samples positive for *E. coli* O157:H7 upon entering the feedlot originated from two of the eight farms providing sheep for this study. Subsequently, throughout the feeding period, only one animal not originally testing positive or from one of the two farms producing *E. coli* O157:H7–positive sheep had a culture-positive fecal sample. Overall, the percentage of fecal samples positive for *E. coli* O157:H7 averaged 9%. Wool samples positive for *E. coli* O157:H7 were found on sheep representing all farms but one, averaging 18% positive overall. Only one carcass swab was *E. coli* O157:H7 positive.

Salmonella-positive fecal samples were detected in sheep from multiple farms of origin. Overall, 11 (7%) of 155 fecal samples were *Salmonella* positive. One lamb not shedding *Salmonella* upon entering the feedlot was later detected shedding *Salmonella* in the feces. *Salmonella* was detected in nearly 50% of the wool samples collected prior to slaughter, while none of the carcasses were *Salmonella* positive 24 h postslaughter (Table 1).

Results of the antimicrobial susceptibility screening are presented in Table 2 by collection date and isolate source (feces, wool, or carcass). All isolates were susceptible to ceftiofur, enrofloxacin, and trimethoprim-sulfamethoxazole (data not shown). Fecal coliforms isolated from sheep upon entry to the feedlot were susceptible to all antimicrobials with the exception of chlortetracycline and oxytetracycline, for which there were two and three resistant isolates, respectively. Similarly, *E. coli* O157:H7 and *Salmonella* iso-

TABLE 2. *Antimicrobial resistance profiles and multidrug resistance of fecal coliforms,* E. coli *O157:H7, and* Salmonella *cultured from fecal, wool, and carcass swab samples from feedlot lambs over four collection periods*[a]

Drug and resistance profile	No. of isolates resistant to indicated drug																	
	27 June			12 August				22 September				27 October						
	Fecal			Fecal			Wool	Fecal		Wool		Fecal			Wool		Carcass	
	FC (n = 20)	EHEC (n = 9)	SALM (n = 7)	FC (n = 20)	EHEC (n = 4)	SALM (n = 1)	SALM (n = 1)	FC (n = 20)	EHEC (n = 1)	EHEC (n = 7)	SALM (n = 11)	FC (n = 20)	EHEC (n = 2)	SALM (n = 2)	SALM (n = 13)	EHEC (n = 2)	EHEC (n = 1)	
Ampicillin	0	0	0	4	0	0	0	2	0	7	4	7	0	1	8	2	0	
Apramycin	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	
Chlortetracycline	2	1	0	16	0	0	1	14	1	4	7	20	2	2	10	1	1	
Florfenicol	0	0	0	0	0	0	0	3	0	4	3	6	0	0	2	1	0	
Gentamicin	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	
Neomycin	0	0	0	1	0	0	0	1	1	0	4	5	0	1	7	0	1	
Oxytetracycline	3	1	0	20	1	0	1	17	1	5	4	20	2	2	8	0	1	
Spectinomycin	0	0	5	2	1	1	0	9	1	6	11	6	0	2	13	0	1	
Sulfachloropyridazine	0	0	0	5	0	0	1	1	1	2	4	4	0	1	7	0	1	
Sulfadimethoxine	0	0	0	5	0	0	1	1	1	2	4	2	0	1	8	0	1	
Sulfathiazole	0	0	0	5	0	0	1	1	1	2	4	3	0	1	8	0	1	
Resistant to:																		
0 antibiotic	17	8	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	
1 antibiotic	1	0	5	4	2	1	0	4	0	0	3	0	0	0	2	1	0	
2 antibiotics	2	1	0	9	0	0	0	7	0	1	2	7	2	0	2	0	0	
≤4 antibiotics	0	0	0	2	0	0	0	8	0	2	2	6	0	1	4	1	0	
≤6 antibiotics	0	0	0	4	0	0	1	1	0	3	0	5	0	0	0	0	0	
≤8 antibiotics	0	0	0	0	0	0	0	0	1	1	4	2	0	1	3	0	1	
<10 antibiotics	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	

[a] FC, fecal coliforms; EHEC, *E. coli* O157:H7; SALM, *Salmonella*.

lates from the first collection showed little resistance to the antimicrobials examined. However, beginning with the second collection and observed in all subsequent collections, the number of antibiotics to which fecal coliforms were resistant increased, with all isolates resistant to at least one antibiotic, and many of the isolates being multiresistant. Resistance patterns in fecal *E. coli* O157:H7 isolated in later collections were largely similar to those of the first collection. One *E. coli* O157:H7 isolate that was cultured from a carcass swab in the last collection period was resistant to seven antibiotics. Seven wool *E. coli* O157:H7 isolates were also collected at this same time, all of which were multidrug resistant. *Salmonella* isolates from the feces and wool were increasingly antibiotic resistant as the sample collections progressed. Two fecal *Salmonella* isolates cultured during the last collection were resistant to three and eight antibiotics each, while wool isolates ($n = 13$) from this same collection ranged in resistance from one to nine antibiotics.

DISCUSSION

To our knowledge, this is the first report concerning the prevalence of *E. coli* O157:H7 and *Salmonella* in feedlot sheep in the United States. Previous research has documented that sheep, like cattle, are naturally colonized by *E. coli* O157:H7 *(4, 6, 13)*; however, these studies utilized grazing animals and/or were conducted outside the United States. In the present research, the prevalence of fecal *E. coli* O157:H7 (9%) and that of *Salmonella* (7%), when averaged across collection times, were generally lower than previously reported figures for feedlot cattle *(2, 7, 10, 12)*. In the case of *E. coli* O157:H7, all the lambs with positive fecal samples originated from one of two farms and only one lamb not shedding upon entering the feedlot was detected shedding this pathogen at a later sample date. Fecal *Salmonella* was detected in lambs from multiple farms originally, and similar to what was observed for *E. coli* O157:H7, only one lamb not shedding *Salmonella* upon entering the feedlot was detected shedding during the feeding period.

The percentage of wool samples collected prior to slaughter and positive for *E. coli* O157:H7 was higher than for fecal samples, and these positive wool samples were equally distributed among lambs from all farms. The one exception is farm F, which had the highest percentage of lambs with positive fecal samples but no wool samples positive for *E. coli* O157:H7. Most likely the wool was contaminated with *E. coli* O157:H7 in the feedlot; however, as we did not collect wool samples from lambs upon their entering the feedlot, it is possible that the wool was infected prior to feedlot entry. *Salmonella* was detected on a greater percentage of wool samples (49%) than *E. coli* O157:H7 (18%), although fecal shedding of the two pathogens was similar. At least one lamb representing each farm of origin had a *Salmonella*-positive wool sample. The reason for the higher percentage of *Salmonella*-positive wool samples is unknown. Quantitative enumeration of this pathogen may have explained this difference, but only qualitative analysis of the samples was conducted. These findings are not surprising, as hides have been implicated as a major source of potential carcass contamination in feedlot cattle *(12)*. The good news is that while approximately one-half of the lambs entering the slaughter facility were detected with *E. coli* O157:H7 or *Salmonella* on the wool, in-plant processing methodology and carcass washing procedures in this facility were effective in preventing cross-contamination, with only one carcass swab found to be positive for *E. coli* O157:H7.

Similar to prevalence reports of pathogenic bacteria in feedlot sheep, there is a scarcity of information in regard to their antimicrobial resistance profiles. This is not to say that this type of information is not available for sheep; however, the majority of the data relate to isolates from clinical cases and not isolates cultured from apparently healthy animals as in the current research. Due to the limited number of *E. coli* O157:H7 and *Salmonella* isolates, we also cultured and randomly selected 20 fecal coliform isolates for antimicrobial susceptibility screening on each of the collection dates. In general, we observed an increase in the number of isolates showing resistance to one or more antibiotics, as well as the number of multiresistant isolates, as the feeding period progressed. Multidrug resistance was observed in this study; however, as might be expected, the patterns of resistance were to antimicrobials commonly used in veterinary medicine (ampicillin, tetracyclines, and sulfonamides) and not those used in human medicine. All isolates examined were susceptible to enrofloxacin and ceftiofur, antibiotics that are indicated for the treatment of salmonellosis in humans.

Results of this research demonstrate that feedlot sheep, like cattle, are naturally colonized with the foodborne pathogens *E. coli* O157:H7 and *Salmonella* and that the wool could be a significant potential source of carcass contamination. Furthermore, while in-plant processing procedures and intervention strategies are largely effective in preventing carcass contamination, the implementation of preharvest intervention strategies will be an important part of a multihurdle approach to food safety.

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